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Increased feeding damage under elevated Co_2: The case of the Russian wheat aphid

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ABSTRACT

We report the results of a baseline study on the effects of Russian wheat aphid infestation on barley lines grown under ambient and elevated (450 and 550 μmol mol^{-1}) CO_2 concentrations [CO_2]. Elevated CO_2 impacted on plant biomass, C:N ratios and leaf nitrogen concentrations. Visible manifestation of aphid feeding related damage was assessed by examining resultant chlorosis and leaf roll under ambient and two elevated [CO_2] levels using a control and three resistant barley host combinations. Elevated [CO_2] had a significant positive effect on the growth of the four barley lines that were not infested by the aphids. However under the same conditions aphid feeding under elevated CO_2 conditions caused very high biomass loss, which was more noticeable in experiments involving non-resistant PUMA than in the resistant barley lines. The results of this study demonstrate that CO_2 enrichment substantially increases aphid populations of RWASA1 and RWASA2 on the four barley lines investigated. Furthermore, aphid populations were higher on non-resistant PUMA than the three resistant lines and the RWASA2 biotype out-performed RWASA1 in each case. Under elevated [CO_2], aphid feeding, resulted in a significant increase in the leaf C:N ratios (as a percentage change) in most treatments, compared to levels recorded on uninfested plants. The resistant lines also showed a significant reduction in leaf nitrogen (~40% for PUMA and not less than 30% for the resistant STARS lines tested). C:N ratio changes and N loss correlated to [CO_2] and aphid biotype. By 28 days of infestation, most of the non-resistant PUMA line in particular showed significant irrecoverable levels of leaf chlorosis. At level 9 rating on the chlorosis scale (i.e. plant death when recovery was not possible), experiments were terminated. As aphid success is unlikely to be the sole product of [CO_2], but also of other limiting nutrients such as N, it may be worth further investigating the effect of plant quality and ultimately plant nutrition on the population growth of aphids.

1. Introduction

Anthropogenic activities have resulted in an alarming amplification of the rate at which concentrations of carbon dioxide ([CO_2]) in the atmosphere are increasing (IPCC, 2010). These activities include fossil fuel consumption, increased industrial activities and deforestation. [CO_2] have risen from ~285 ppm during the Industrial Revolution of 1750 to ~385 ppm in 2005 (Ryan et al., 2010; Stiling and Cornelissen, 2007) and they are expected to continue to rise well into the next century, to above 500 ppm depending on the magnitude of global economic growth and energy use (IPCC, 2010). It has been postulated that the current level of CO_2 in the atmosphere will double within the next century, a scenario that is likely to affect plants from the species to the ecosystem level (Bezemer et al., 1998).

Several studies have shown that [CO_2] influence both photosynthetic and developmental processes in plants (Bassi et al., 1976; Hicklenton and Jollife, 1980). Many C_3 plants grown at elevated [CO_2] attain higher photosynthetic rates and thus faster growth (Hughes and Bazzaz, 1997; Owensby et al., 1999). Barley (*Hordeum vulgare* L.), for example, is reported to grow faster and produce greater yields at elevated CO_2 than at ambient CO_2 concentrations (Sæbø and Mortensen, 1996). Growth at elevated CO_2 levels can result in a large increase in plant biomass due to the accumulation of both structural and non-structural carbohydrates and under nutrient limited conditions may lead to an increase in the C:N ratio as a result of the relative reduction in the nitrogen content of foliage (Bezemer and Jones, 1998; Cotrufo et al., 1998; Lindroth et al., 1995; Poorter et al., 1997). This [CO_2] induced change in plant C:N ratios and nitrogen concentrations is likely to affect plant quality as well as the feeding pattern and behaviour of herbivores (Hughes and Bazzaz, 2001). The aim of this study is to investigate the effect of a CO_2-enriched atmosphere on phloem feeding herbivores such as aphids.

Aphids inflict direct damage on their host plants by removing large quantities of sap and when feeding, are strong secondary sinks (see discussion in Botha and Matsiliza, 2004; Nielsen et al., 1990; Saheed et al., 2010) and inflict indirect damage by serving as virus vectors (Risebrow and Dixon, 1987). Their parthenogenetic reproduction often results in an increased regeneration rate if conditions are favourable (Dixon, 1998). A sustained high breeding rate, or alternatively, if it is increased under conditions under elevated CO_2 levels, may exacerbate plant damage (Harrington et al., 1995). The relationship between CO_2 levels and aphid feeding and breeding rates, may therefore become important,

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and must be considered when examining plant acclimation responses under elevated CO_2 (Rogers et al., 1995).

Many studies on plant–aphid interaction at elevated [CO_2] (e.g. Newman et al., 1999; Newman, 2003; Peltonen et al., 2006; Sudderth et al., 2005; Sun and Ge, 2010) have focussed attention only on aphid performance, without considering the effect of feeding under elevated CO_2 and specifically, on the host plant and its responses. Questions raised about the aphids' potential increased pest performance under elevated [CO_2], with respect to reported changes in plant quality in given herbivory relationships (Ehleringer et al., 2002), need to be addressed. Of particular interest are a number of studies in which aphid-induced reduction in host plant productivity at elevated [CO_2] has been reported (see Awmack and Harrington, 2000; Flynn et al., 2006; Himanen et al., 2008; Hughes and Bazzaz, 2001). These reports suggest that aphid infestation may negate the (expected) beneficial effects of plant growth and productivity at elevated [CO_2].

The Russian wheat aphid (RWA, *Diuraphis noxia* Kurdjumov) is an important pest of small grains such as wheat and barley and has not previously been used in experiments to determine the effect of elevated [CO_2] on these plants to date. We therefore examined the effects of two of the Russian wheat aphid biotypes (RWASA1 and RWASA2) feeding on a non-resistant South African barley variety (Puma) and three USDA varieties (STARS-0502B, 9301B and 9577B) grown under controlled environment conditions, using three CO_2 concentrations (380, 450 and 550 μmol mol^{-1}) grown under non-limiting nutrient conditions. Specifically we hypothesised that:

1. Under non-limiting nutrient conditions and elevated [CO_2], uninfested barley (control plants) will attain higher growth rates, biomass and quality (as reflected by nitrogen content and C:N ratios).
2. Under elevated [CO_2], aphid population growth and size will increase with barley biomass and quality.
3. Enhanced aphid population growth and feeding will negate enhanced barley growth under elevated [CO_2] and will impact on plant biomass, quality (as reflected by nitrogen content and C:N ratios) and survival (as reflected by leaf roll and chlorosis).
4. Aphid effects on barley will vary with [CO_2], plant variety (in terms of resistance) and aphid biotype (in terms of virulence).

2. Materials and methods

2.1. Aphids and plant materials

Plant materials and cultures of RWASA1 and RWASA2 were obtained from the Agricultural Research Council (ARC), Small Grain Institute, Bethlehem, South Africa. Both maintenance of aphid colonies and preparation of plant materials were carried out as described in Jimoh et al. (2011).

2.2. Experimental conditions

All experiments were carried out in controlled environment cabinets (Conviron S10H, Controlled Environment Ltd., Winnipeg, Manitoba, Canada). Given that this study focussed on the effect of aphid feeding under elevated CO_2 all plants were watered every other day with Long-Ashton nutrient solution (Hewitt, 1966) to obviate compounding effects which could appear due to nutrient stress. Three cabinets, each running at 380 μmol mol^{-1} [CO_2] (ambient level), 450 μmol mol^{-1} and 550 μmol mol^{-1} [CO_2] (elevated levels) were maintained at a day time maximum of 24 °C and 66% relative humidity (RH) and at 22 °C, 60% RH (night), with a 14-h photoperiod. The light source was a combination of fluorescent tubes (F48T12.CW/VHO 1500, Sylvania, Danvers, MA) and frosted incandescent 60 W bulbs (Phillips, Eindhoven, The Netherlands), with a PAR level of 250 μmol^{-2} s^{-1} 30 cm below the light source.

Seedlings were sown one per pot, in 17 cm diameter plastic pots filled with potting soil (2:1:1; garden soil:compost:vermiculite mixture) in a greenhouse maintained at 20–30 °C for one week. The seedlings were sprayed with aerosol pyrethroid insecticide (SC Johnson and Sons (Pty) Ltd., South Africa) to kill any insects that may have colonised them while in the greenhouse and subsequently left for 24 h, prior to moving then to growth cabinets (Conviron EF10-H) where they were grown for two weeks to adjust to the growth conditions described above. After reaching the 2–3 leaf stage, they were manually infested with aphids. To ensure that no nutrient limitation took place, we added half strength Long-Ashton nutrient solution (Hewitt, 1966) 3 times per week for the duration of the experiments.

2.3. Experimental design

Each experimental plant consisted of a growing barley plant enclosed under a ventilated cylindrical transparent plastic isolation cage. A total of 360 plants were reared; ten replicates of each treatment combination, 3 aphid treatments (RWASA1, RWASA2 and an uninfested control), ×4 barley lines (Puma, STARS-0502B, STARS-9301B, STARS-9577B)×3 [CO_2] treatments (ambient, 450 and 550 μmol mol^{-1}). Except for the control plants (which were also enclosed in plastic isolation cages), each plant was infested with either RWASA1 or RWASA2 by placing a leaf segment containing 10 adult apterous aphids in the axil of leaves. The aphids were allowed a 24 h period to transfer and acclimatize to the experimental plants before commencing measurements of the effect of treatments. Experimental plants under each [CO_2] treatment (10×3 aphid treatments×4 barley lines = 120 plants) were arranged in the growth cabinet using a complete randomized block design. Experimental procedures were conducted twice.

2.4. Effects of [CO_2] on aphid population growth

Populations of aphids on each plant were assessed at days 1, 7, and 14 after infestation (DAI) for each of the three [CO_2] levels. The adaxial and abaxial surfaces of every leaf on each plant were carefully examined and numbers of aphids were non-destructively counted with the aid of a hand lens on each of these days. Data for each [CO_2] level were separately analysed using STATISTICA 9. Aphid types (2), barley lines (4) and days of infestation (3) were the independent variables while the number of aphids constituted the dependent variable. Prior to analysis, homogeneity of variances and normality of the aphid population data were examined using Levene's and Shapiro–Wilk's tests respectively (Johnson and Wichern, 2002). Statistical significance was determined using repeated measures of ANOVA design at 5% level of significance.

2.5. Effects of [CO_2] and aphids on plants

2.5.1. Virulence of aphids on plants

Virulence of the two biotypes on infested plants were measured in forms of chlorosis and leaf roll at 1, 7, 14, 21 and 28 DAI using a chlorosis scale where 0 was healthy with no chlorosis or necrosis and 9 was scored where leaves and or plant death was obvious. Leaf rolling was scored 1 where no rolling was evident to 3 where leaves were tightly/completely rolled (see Jimoh et al. (2011)). Data were analysed for each symptom category under each [CO_2] level as described above for population growth except that each symptom category was classified as a dependent variable.

2.5.2. Plant biomass

After 28 days of infestation, when most of the non-resistant PUMA line showed level 9 rating on the chlorosis scale (i.e. plant death when-recovery was not possible), experiments were terminated. Plant material in each pot was carefully removed. The root system was washed of soil by soaking the root mass in a large volume of water and using screen mesh to recover loose roots (Reid and Fiscus, 2008). The entire vegetative material

was enclosed in a medium-sized paper envelope and oven-dried at 60 °C for 48 h. Dried plant material was weighed to obtain total dry biomass.

2.5.3. Nitrogen concentration and C:N ratio of leaves

Five plants, out of the ten replicates, were randomly selected for determination of nitrogen concentrations and C:N ratios of leaves. 3 cm-long leaf segments were cut from the mid-leaf region of every leaf from each of the three treatments (i.e. uninfested, RWASA1, RWASA2). In the case of infested plants, leaf surfaces were carefully brushed using a fine paintbrush to guard against contamination from aphids or its parts. Leaf segments from each sample were ground and homogenised to a fine powder in a mortar and pestle that was cleaned between samples. Ground sample was packed and stored in 1.5 ml polyvinyl Eppendorf tube and further desiccated for 48 h. 1.45–1.65 mg of the powder was weighed out into clean 9×5 mm OEA tin capsule using an analytical semi-micro weighing balance (OHaus Discovery DV 215CD, Ohaus Corporation, Switzerland). Tin capsules were crimped and gently folded repeatedly into a compact ball and then stored in 96-cell well culture plates before analysis. The samples were analysed for both %nitrogen g^{-1} and C:N ratio of leaf tissues using a Europa Scientific Elemental Analyser (Model ANCA-SL, Europa Scientific, United Kingdom). Data for each measurement were separately analysed for each [CO_2] level on STATISTICA 9 using factorial ANOVA design at 5% level of significance. Infestation treatment (×3), and barley lines (×4) were the independent variables while % nitrogen or C:N ratio constituted the dependent variable.

3. Results

3.1. Effect of [CO_2] on biomass components

Elevated [CO_2] had a significant positive effect on the growth of the four barley lines that were not infested by the aphids. The total biomass of each of the four barley lines increased significantly under the two elevated [CO_2] levels when compared to their respective values at ambient [CO_2] level (Fig. 1). The results showed that increase in biomass in each barley line is proportional to [CO_2] increase, as the greatest increase was recorded in plants grown at 550 μmol mol^{-1}.

The data shown in Fig. 1 clearly show that there was a significant loss in biomass as a result of RWA feeding on all four barley lines, under ambient as well as the two elevated CO_2 levels. The two biotypes caused greater reduction in the biomass of the non-resistant PUMA line than on any of the three resistant lines, with the least loss (63%) recorded for RWASA1 feeding and the greatest (82%) by RWASA2 at 450 μmol mol^{-1}. In general terms, aphid feeding under elevated CO_2 conditions caused very high biomass loss.

This is highlighted in Table 2, where biomass reduction was expressed as a percentage of the uninfested control plants. Interestingly, the loss in biomass in the resistant STARS lines under 450 μmol mol^{-1} CO_2 caused as a result of RWASA1 feeding, was not dissimilar to that calculated for the non-resistant PUMA plants. It is clear that at all [CO_2] levels, that RWASA2 caused greater reduction in total biomass than did RWASA1. The data reflect a trend in the percentage reduction of total biomass suffered by PUMA infested by either RWASA1 or RWASA2 among the three [CO_2] levels that was 450 > 550 > ambient (Table 1).

3.2. Effect of [CO_2] on total leaf nitrogen

When expressed as reduction of leaf N, (Table 2) the results, while variable, show greater N loss when RWASA2 fed, than was the case with RWASA1. In uninfested plants of the four lines, leaf nitrogen concentration increases with [CO_2]. However, feeding by the two RWA biotypes on the four barley lines resulted in a significant depletion of nitrogen concentration of leaves, in all [CO_2] treatments (Fig. 2). The two aphid biotypes clearly depleted leaf nitrogen content of both resistant and non-resistant plants. Examination of the data presented in Table 2 suggests that but for one exception (24%; STARS-0502B at ambient [CO_2] level). RWASA2 caused a greater % reduction of leaf nitrogen than RWASA1 under all three CO_2 levels and four barley lines (Table 2).

3.3. The effect of elevated CO_2 on leaf C:N ratio

Of the four lines, only STARS-9301B showed a consistent (but insignificant) increase in C:N ratios with increasing [CO_2] under control conditions. In contrast, feeding by the two RWA biotypes, caused a considerable increase in the leaf C:N ratios in all four barley lines,

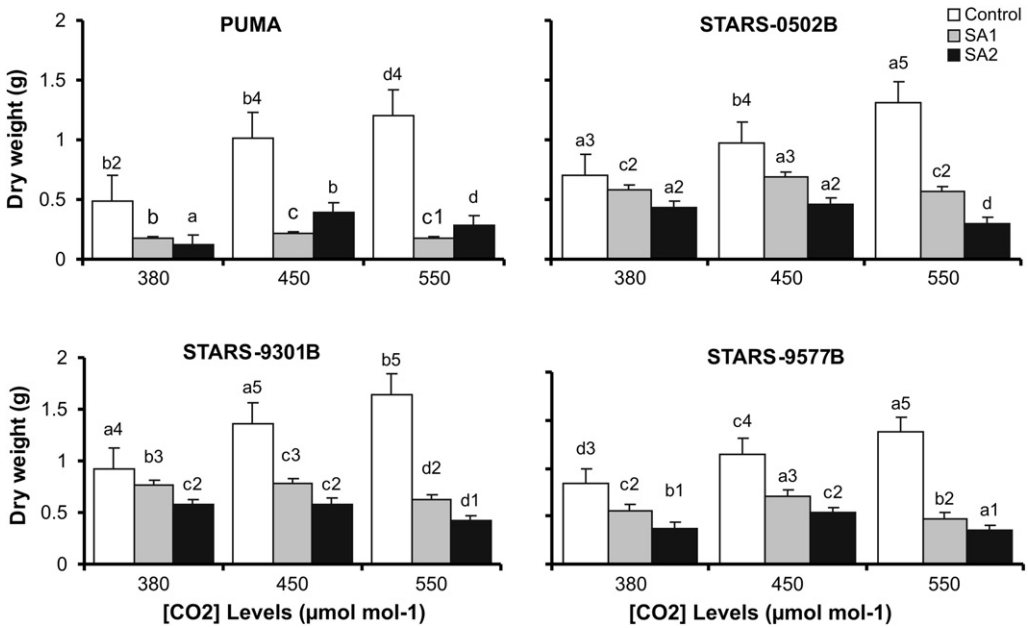

Fig. 1. Total biomass [dry weight (g)] of control and infested plants grown under ambient and elevated [CO_2]. Bars with different letters and numbers indicate significantly different homogenous groups at 0.05 level using Tukey's posthoc test (n = 10).

Table 1
Reduction in the total biomass of barley lines infested with RWASA1 and RWASA2 at the three levels of [CO_2] expressed as a percentage of the values for uninfested plants.

Barley lines	Ambient (%)		450 μmol mol^{-1} (%)		550 μmol mol^{-1} (%)	
	RWASA1	RWASA2	RWASA1	RWASA2	RWASA1	RWASA2
PUMA	63	76	79	82	68	77
STARS-0502B	18	38	29	53	57	77
STARS-9301B	16	36	42	57	62	74
STARS-9577B	32	54	38	54	65	75

when compared to levels recorded on uninfested plants, under each of the three [CO_2] levels (Fig. 4).

3.4. Aphid damage to host plants at varying levels of [CO_2]

All four barley lines grown under the three CO_2 levels showed visible feeding-related symptoms associated with RWA infestation on host plants, for RWASA1 and RWASA2. These symptoms include leaf chlorosis, necrosis, longitudinal streaks on leaves, as well as leaf rolling, with chlorosis and leaf rolling being the principal visible criteria used to evaluate damage on host plants during infestation (see Burd et al., 1993; Jimoh et al., 2011 and Puterka et al., 2006).

3.4.1. Chlorosis

By day 7, chlorosis symptoms became more obvious with exposure to feeding aphids. All four barley lines grown under elevated CO_2 were more adversely affected under elevated CO_2, compared to ambient levels as from 7 DAI (Table 3). By day 14, non-resistant plants displayed extensive chlorosis symptoms at all three CO_2 levels and by 21 DAI, some non-resistant plants infested with RWASA2. By 28 DAI chlorosis was extensive and some plants had died. Interestingly, STARS-0502B appeared less resistant to RWASA2 than did STARS-9301B or -9577B under both elevated [CO_2] (Table 4).

3.4.2. Leaf roll

Under control (380 ppm CO_2) leaves of the non-resistant PUMA and STARS-0502B (a resistant line) infested by the two aphids, were more evident and appeared loosely folded by 7 DAI (Table 4). Leaf folding increased more noticeably and by 28 DAI rolling was more pronounced on the control and STARS-0502B plants. This trend was apparent across the four [CO_2] as well (see Table 4). The data presented in Table 4 shows that RWASA2 caused more obvious leaf roll symptom damage than RWASA1 at all [CO_2] levels which confirms this biotypes increased virulence under elevated CO_2.

3.5. Effects of [CO_2] on the population growth of RWASA1 and RWASA2

Populations of RWASA1 and RWASA2 on each of the four barley lines increased progressively with days of infestation, irrespective of the level of [CO_2] (Fig. 3A–D). As expected, RWA population sizes were significantly higher on non-resistant PUMA than the mean of their respective populations on the three resistant lines ($p<0.01$). Irrespective of the barley line, [CO_2] level and days of infestation, RWASA2 bred faster than RWASA1 (Fig. 3A–D).

Independent of aphid biotype, barley line or days of infestation, aphid populations at the two elevated [CO_2] were significantly larger than their populations at ambient [CO_2] ($p<0.01$). At ambient [CO_2], populations of the two biotypes were significantly larger on the non-resistant PUMA than the average recorded for the three resistant lines. RWASA2 bred faster than RWASA1 in each case. However, a comparison of aphid populations at each of the two elevated [CO_2] gave variable results between the two biotypes (Fig. 3). The trend of their population growth on each of the four barley lines is 14d > 7d > 1d. Among the resistant lines, STARS-0502B bred less aphids than both STARS-9301B and STARS-9577B across the three [CO_2] levels after 1 and 7 DAI.

Table 2
Reduction in the leaf nitrogen concentration of barley lines infested with RWASA1 and RWASA2 at the three levels of [CO_2] expressed as a percentage of the values for uninfested plants.

Barley Lines	Ambient (%)		450 μmol mol^{-1} (%)		550 μmol mol^{-1} (%)	
	RWASA1	RWASA2	RWASA1	RWASA2	RWASA1	RWASA2
PUMA	25	41	16	25	34	40
STARS-0502B	26	24	22	49	30	47
STARS-9301B	18	25	26	37	29	33
STARS-9577B	11	22	18	42	23	30

4. Discussion

As expected, all uninfested plants grown at elevated [CO_2] had a higher biomass at the termination of the experiments, compared with those grown at ambient concentrations (Fig. 1). The data presented here are therefore in agreement with previous studies that have reported an increase in plant biomass at elevated [CO_2] (Awmack and Harrington, 2000; Hughes and Bazzaz, 2001; Mondor et al., 2005; Newman et al., 1999) and support the concept of accelerated plant growth with increased CO_2 availability (Barbehenn et al., 2004; Flynn et al., 2006; Reich et al., 2006). At elevated CO_2 levels, the gaseous CO_2 in the immediate plant environment increases (Schlesinger, 1997), with more CO_2 available for absorption into the leaf, as increasing the [CO_2] creates a steeper air-leaf mesophyll CO_2 gradient which facilitates the entry of a higher concentration of [CO_2] through stomata into the leaf (Rowland-Bamford et al., 1991), thereby favouring photosynthetic carbon reduction over oxygenation of ribulose-1, 5-bisphosphate carboxylase/oxygenase (see Drake et al., 1997; Stitt, 1991). This leads in turn to increased carbohydrate synthesis (Conroy et al., 1994; Rogers and Dahlman, 1993; Woodrow, 1994).

Concomitant with an increase in biomass, uninfested plants grown under elevated [CO_2] had an increased percentage foliar nitrogen concentration (Fig. 2). There are several reports that argue that carbon dioxide enrichment induces a decline in leaf nitrogen (Cotrufo et al., 1998; Dixon et al., 1993; Hughes and Bazzaz, 2001; Stiling and Cornelissen, 2007). It is important to note that most of these studies were however, conducted using either open-topped chambers (Morgan et al., 2001; Newman et al., 1999), or techniques of Free-Air Carbon dioxide Enrichment (FACE) (see Awmack et al., 2004; Taub, 2010). Stitt and Krapp (1999) suggested that photosynthetic responses and growth under elevated [CO_2] will depend on the availability of inorganic nutrients and their utilization by the plants. Riviere-Rolland et al. (1996) showed that a decrease in leaf nitrogen is to be expected in plants grown under conditions of limited nitrogen supply, but not when the plants are supplied with abundant nitrogen in the form of nitrates. Adequate nitrogen supply (as was the case in this study), should thus not result in a major decrease in the internal nitrogen concentration, or of the levels of nitrogen metabolites that would be expected under enriched [CO_2]. It follows that increased rates of growth (as a result of increased synthesis of carbohydrates under elevated [CO_2]) must require higher rates of inorganic nitrogen uptake and subsequent assimilation into plant tissues (Stitt and Krapp, 1999). Unlike previous studies, where N nutrients may have been limiting, in this study, regular application of Long Ashton nutrient solution (Hewitt, 1966) contributed to the increased uptake, storage and assimilation of N into plant tissues.

As previously mentioned, uninfested barley plants increased in biomass with increasing [CO_2], but, in all cases, aphid infestation negated this increase in plant growth. The reduction in biomass of aphid-infested plants was most pronounced on non-resistant PUMA compared with the three resistant lines (Table 1). We noted that RWASA2 caused more damage (due to its faster population growth which contributed to a pronounced reduction in plant biomass) than did RWASA1.

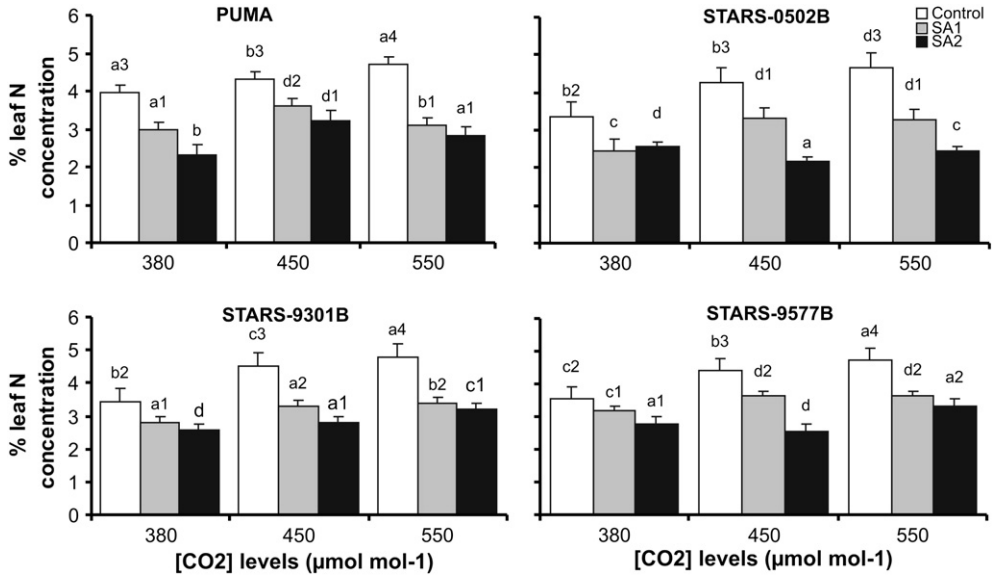

Fig. 2. Percentage foliar nitrogen concentrations (g^{-1} of leaf tissue) of control and infested barley leaves, under three levels of [CO_2] at 28 DAI. Bars with different letters and numbers indicate significantly different homologous groups at 0.05 level using a Tukey's posthoc test (n = 5).

Awmack and Harrington (2000) showed that when *Vicia faba* was infested with the pea aphid, *Acyrthosiphon pisum*, a reduction in shoot fresh weight at both ambient and elevated [CO_2] levels was evident. A number of investigations (Docherty et al., 1996; Flynn et al., 2006; Hughes and Bazzaz, 2001; Johnson and Lincoln, 1990; Lincoln et al., 1986) have also reported that aphid-infested plants suffer a substantial reduction in total biomass at both ambient and elevated [CO_2]. Furthermore, the data presented here are consistent with those for chewing herbivorous insects where damage by the insects increases at elevated [CO_2] (see Lincoln et al., 1986; Johnson and Lincoln, 1990; Docherty et al., 1996). Clearly, biomass reduction could result from diversion of assimilates (aphids are strong diversionary sinks) as well as the increased aphid/insect population. An increase in aphid population size could exacerbate the inter-aphid competition for a suitable feeding site. This competition could explain why Sun and Ge (2010) hypothesised that aphids could spend more time, probing and ingesting sap from leaves under elevated [CO_2] than they do under ambient CO_2 conditions. Increased probing, would conceivably result in additional visible leaf damage effects.

Leaf nitrogen concentration loss (see Fig. 2) is we feel, due to the specific requirements for certain amino acids present in phloem sap (see Risebrow and Dixon, 1987). Nitrogen is a crucial factor in insect herbivore diets (Mattson, 1980). Given that phloem sap is generally low in protein (Douglas, 1993), then any change in the nitrogen content in host plant (as a result of elevated CO_2, or due to depletion of nitrogen for example) will impact on herbivore feeding pattern and behaviour (see Bezemer and Jones, 1998). It follows thus that the N content of plant tissues may become a limiting resource for the growth, development and performance of feeding aphid populations (Bezemer and Jones, 1998). Increased feeding intensity (due to population

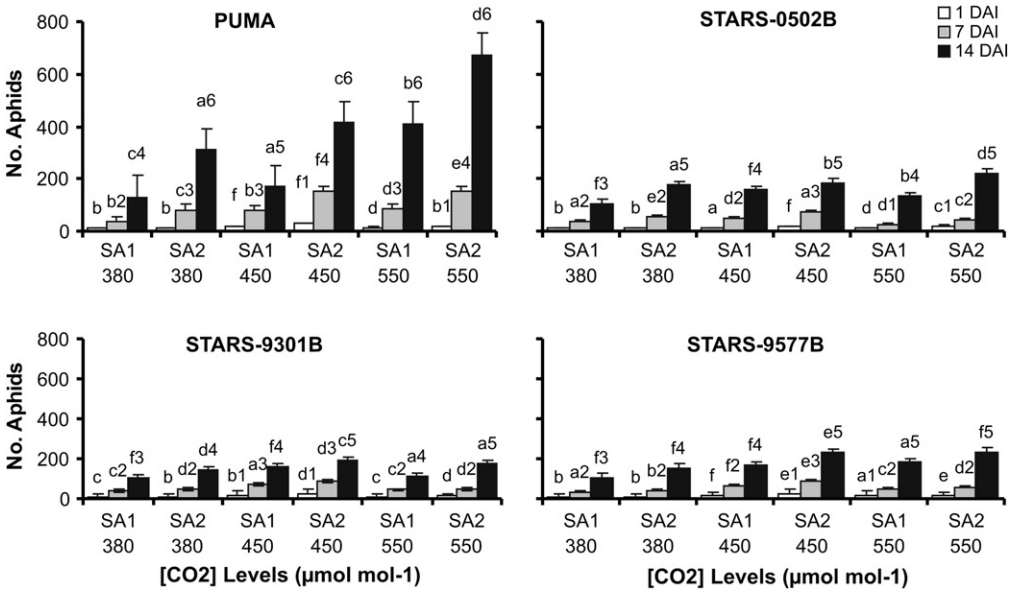

Fig. 3. Population growth of RWASA1 and RWASA2 on the four barley lines at 1, 7 and 14 DAI under the three levels of [CO_2]. Bars with different letters and numbers indicate significantly different homogenous groups at 0.05 level using a Tukey's posthoc test (n = 10).

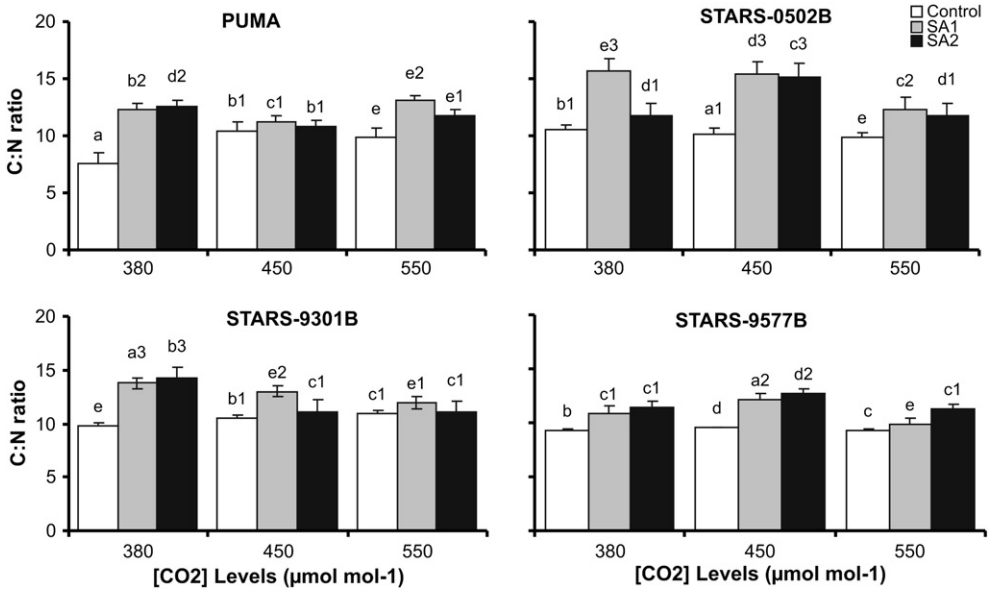

Fig. 4. C:N ratios in control and infested barley leaves grown under ambient and elevated [CO_2]. Bars with different letters and numbers indicate significantly different homogenous groups at 0.05 level using a Tukey's posthoc test (n = 10).

pressure) would thus impact on and reduce available protein (the N-pool) of the plants.

The C:N ratios recorded in this study were variable. Other studies indicate that an increase in the rates of photosynthesis at elevated [CO_2] leads to an increase in C:N ratio of plants (see Hughes and Bazzaz, 2001; Lindroth et al., 1995; Stiling and Cornelissen, 2007). Any increase in aphid feeding, for example due to a greater population size under elevated [CO_2], would deplete the nitrogen content of the host and would impact on the C:N ratios.

Elevated [CO_2] affect plant growth, as well as the population growth of the two RWA biotypes used in this study, lending agreement to the study by Mondor et al. (2005) who reported that elevated [CO_2] positively affect the population abundance of aphids. The increase in net photosynthetic capacity of plants, resulted in an increase in biomass under enriched CO_2 conditions, which in turn resulted in more assimilates being immediately available to the feeding aphids. We observed that this impacted positively on the breeding capacities and feeding behaviour of the two aphids, which are higher at elevated CO_2 levels than under ambient CO_2. The ultimate outcome was early plant death — usually around 28–30 DAI. Our data essentially agrees with that on *Macrosiphum euphorbiae* (Flynn et al., 2006), on *Myzus persicae* (Bezemer et al., 1998, 1999; Hughes and Bazzaz, 2001) and on *Aphis rumicis* (Whittaker, 1999).

In contrast, other studies have provided varying and inconsistent results. In a five aphid-plant interaction study, Hughes and Bazzaz (2001) reported that elevation of [CO_2] negatively affected population growth of *Acyrthosiphon pisum*, positively affected that of *M. persicae* and had no significant effect on those of *Aphis nerii*, *A. oenotherae* and *Aulacorthum solani*. Docherty et al. (1997) also reported inconsistencies in the response of aphid performance at elevated [CO_2]. However, results of this study showed that CO_2 enrichment substantially increased the populations of RWASA1 and RWASA2 on the four barley lines. It also showed that aphid populations were higher on non-resistant PUMA than the three resistant lines and that RWASA2 out-performed RWASA1 in each case. As aphid success is unlikely to be the sole product

Table 3
Mean ratings for chlorosis of barley lines infested with RWASA1 and RWASA2 at the three CO_2 levels (n = 10).

DAI	Barley line	380 µmol mol^{-1}		450 µmol mol^{-1}		550 µmol mol^{-1}	
		RWASA1	RWASA2	RWASA1	RWASA2	RWASA1	RWASA2
1	PUMA	0.10a	0.10a	0.00a	0.00a	0.00a	0.00a
	STARS-0502B	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
	STARS-9301B	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
	STARS-9577B	0.00a	0.00a	0.00a	0.00a	0.00a	0.00a
7	PUMA	1.30c	1.80f	3.00e1	4.70c3	1.30c	2.10a1
	STARS-0502B	1.00b	1.00b	3.90e2	5.00e3	2.50d1	2.90e1
	STARS-9301B	1.00b	1.00b	1.40d	1.70e	1.00b	1.20c
	STARS-9577B	1.00b	1.00b	1.70e	1.90f	1.00b	1.00b
14	PUMA	6.70e4	7.20c5	5.50a4	7.10b5	4.60b3	6.70
	STARS-0502B	4.20e2	3.20a2	6.10b4	6.70e4	3.70d2	4.70b3
	STARS-9301B	3.90c2	2.20b1	3.00e1	4.00e2	2.40c1	1.90f
	STARS-9577B	3.10f1	3.10f1	3.10f1	4.40a3	1.90f	2.50d1
21	PUMA	7.70a6	8.70e6	7.80b6	8.70e6	6.40c4	8.60e6
	STARS-0502B	6.80f4	4.70c3	7.60f5	8.50e6	5.40f3	7.50e5
	STARS-9301B	7.20c5	3.00f1	5.40f3	6.40c4	3.50b2	3.80d2
	STARS-9577B	6.90a5	4.30e2	4.80d3	6.40c4	3.40b2	4.60f2
28	PUMA	8.70e6	9.00f6	9.00f6	9.00f6	8.00c6	9.00f6
	STARS-0502B	7.30d5	5.40f3	8.60e6	9.00f6	8.10c6	8.80e6
	STARS-9301B	7.50e5	4.70c3	7.50e5	8.20d6	5.00e3	6.70e4
	STARS-9577B	7.30d5	5.40f3	6.50d4	8.20d6	5.30f3	6.80f4

Values are means of 10 replicates. Values followed by different notations are significantly different following Tukey's posthoc test ($p<0.05$).

Table 4
Mean ratings for leaf roll damage symptoms of barley lines grown under the three CO_2 levels and infested with RWASA1 and RWASA2 (n = 10).

DAI	Barley line	380 μmol mol^{-1}		450 μmol mol^{-1}		550 μmol mol^{-1}	
		RWASA1	RWASA2	RWASA1	RWASA2	RWASA1	RWASA2
1	PUMA	1.00a	1.10a	1.00a	1.00a	1.00a	1.00a
	STARS-0502B	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
	STARS-9301B	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
	STARS-9577B	1.00a	1.00a	1.00a	1.00a	1.00a	1.00a
7	PUMA	1.70a1	2.30f1	1.60f	1.70a1	1.00a	1.50e
	STARS-0502B	1.60f	1.70a1	1.50e	1.80b1	1.30c	1.30c
	STARS-9301B	1.00a	1.70a1	1.00a	1.00a	1.00a	1.00a
	STARS-9577B	1.00a	1.70a1	1.00a	1.00a	1.00a	1.00a
14	PUMA	2.30f1	3.00a3	2.30f1	2.60c2	2.20e1	2.70d2
	STARS-0502B	2.00d1	2.00d1	1.70a1	2.00d1	1.70a1	1.90c1
	STARS-9301B	1.20b	1.90c1	1.30c	1.60f	1.30c	1.30c
	STARS-9577B	1.30c	2.00d1	1.60f	1.70a1	1.20b	1.30c
21	PUMA	2.60c2	3.00a3	2.80e2	3.00a3	2.40a2	3.00a3
	STARS-0502B	2.30f1	2.402a	2.20e1	2.50b2	2.10e1	2.60c2
	STARS-9301B	1.40d	2.00d1	1.90c1	2.00d1	1.40d	1.80b1
	STARS-9577B	1.50e	2.20e1	1.90c1	2.20e1	1.50e	1.80b1
28	PUMA	2.70d2	3.00a3	2.90f2	3.00a3	2.60c2	3.00a3
	STARS-0502B	2.70d2	2.50b2	2.50b2	2.80e2	2.40a2	2.90f2
	STARS-9301B	1.40d	2.30f1	2.50b2	2.70d2	1.70a1	2.30f1
	STARS-9577B	1.70a1	2.60c2	2.20e1	2.70d2	1.90c1	2.40a2

Values are means of 10 replicates. Values followed by different notations are significantly different following Tukey's posthoc test ($p<0.05$).

of [CO_2], but also of other limiting nutrients such as N, it may be worth further investigating the effect of plant quality and ultimately plant nutrition on the population growth of aphids.

Chlorosis and leaf roll are two symptoms that have been identified as important visible criteria that are useful both for evaluating damage caused by RWA (Burd et al., 1993) and for establishing biotypic variation among RWA biotypes in different geographical locations on resistant and non-resistant hosts (Puterka et al., 1992). In this study, development of chlorosis symptom on the plants was gradual and worsened with sustained feeding exposure (Table 3). Chlorosis became noticeable on leaves of all barley lines irrespective of aphid biotype or levels of [CO_2] at 7 DAI. This lends support to Deol et al. (2001) who reported that RWA feeding on host plants required 7d of infestation before chlorosis became noticeable, spreading and worsening thereafter.

4.1. Conclusions

Barley has evolved worldwide and is now an important habitat for many insect pests such as RWA. Although controlled environment experiments could be argued to be of 'limited scope or value' in relation to the field conditions, we remain convinced that baseline experiments such as those reported here are essential, as it is only through the control of temperature, humidity and [CO_2] for example, that it is even remotely possible to predict potential impact and issues related to CO_2 level changes, nitrogen availability, or droughting conditions. Modelling the effects of aphid population growth becomes an extremely difficult exercise to undertake in the field, as climatic factors cannot be controlled. Although the experiments described here are limited to ambient (380 ppm) and 450 and 550 ppm CO_2 levels, we are confident that the results presented here, are realistic, as 450 ppm could quite possibly be reached within 20–30 years. Our controlled environment study demonstrates that feeding by the two RWA biotypes impacted negatively on the plants grown at ambient as well as at elevated CO_2 levels. We argue that changing climate and specifically, elevated CO_2 may well pose a very serious threat to small grain crops' productivity in the future. Though it is evident that RWASA2 causes greater biomass reduction than RWASA1, the fact that population size for both biotypes was suppressed on the resistant lines at elevated [CO_2] levels, suggests that these resistant lines (and others yet to be tested) have the potential to ameliorate the negative effect of denser aphid infestations. However, there is no denying that the combined effects of RWA infestation and elevated CO_2 sound a warning — there is an urgent and critical need for further studies to provide ultra structural and physiological explanations on the effects of elevated [CO_2] on infestation of biotypes of aphids on susceptible and resistant plants to compliment what is known at ambient [CO_2] level. The knock-on effect of increased photosynthesis will be an increase in the aphid population, decreased biomass and this has the potential for early and severe crop loss.

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